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**Research Paper**

Ingestion of plastic by fish: a comparison of Thames Estuary and Firth of Clyde populations

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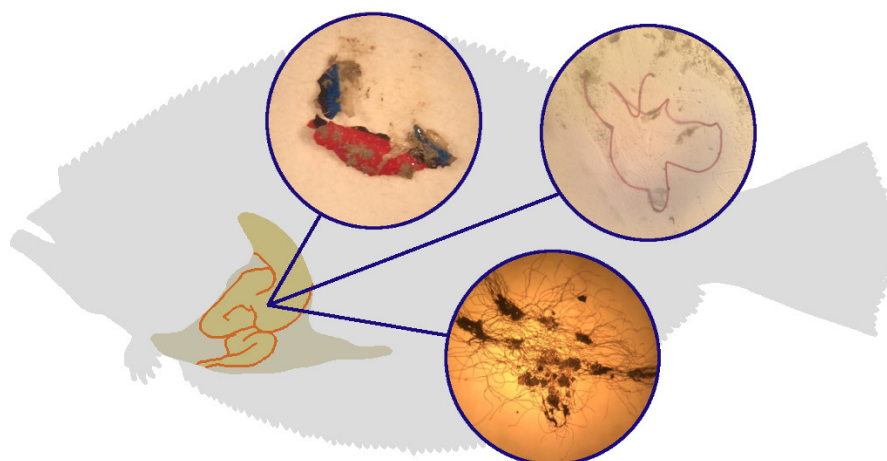
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## ABSTRACT



This study compared plastic ingestion between pelagic and benthic fish populations from two UK watersheds: the Thames Estuary and the Firth of Clyde. The alimentary canals of 876 individuals were examined. Of twenty-one estuarine species investigated, fourteen ingested plastics, including predator (fish) and prey (shrimp) species. Overall, 32% of organisms ingested plastic, mostly fibres (88% of total plastics). More flatfish (38%) ingested plastics than other benthic species (17%). In the Thames, more plastic was ingested by pelagic species (average number of plastic pieces ingested: 3.2) and flatfish (average number of plastic pieces ingested: 2.9) than by shrimp (average number of plastic pieces ingested: 1). More fish from the Clyde ingested plastic than similar Thames species (39% compared to 28% respectively); however, the average amount of plastic ingested did not differ between the sites.

## ARTICLE INFO

### *Keywords:*

Microplastics; Microfibres; Thames Estuary; Firth of Clyde Fish populations; FTIR Spectroscopy

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- 33    Memorial Fund.

## 1. Introduction

Plastic has been mass-produced since the 1940s and is now a huge source of marine pollution world-wide (Galgani et al., 2000; Moore, 2008; Barnes et al., 2009; Browne et al., 2011; Corcoran, 2015; Jambeck et al., 2015). In 2016, 335 million tonnes of plastic were produced globally and production increases yearly (PlasticsEurope, 2018), as does the amount entering the marine environment (Jambeck et al., 2015). In 2010 alone an estimated 12.7 million tons of plastic entered the ocean (Jambeck et al., 2015). Plastic debris has been reported to be ingested by ca. 220 species (Lusher et al., 2017), including marine and freshwater fish (Lusher et al., 2013; Phillips and Bonner, 2015), crustaceans (Murray and Cowie, 2011; Devriese et al., 2015), molluscs (Van Cauwenberghe and Janssen, 2014; Van Cauwenberghe et al., 2015), seabirds (Avery-Gomm et al., 2013) and mammals (Lusher et al., 2015).

It is estimated that, in the marine environment, plastics take hundreds to thousands of years to degrade (Barnes et al., 2009), with Corcoran et al. (2015) reporting the presence of microplastics in lake sediment that had been accumulating for 38 years. Despite this, tide action, photodegradation, biodegradation, thermo-oxidative degradation and hydrolysis can breakdown plastics in the marine environment into ever decreasing smaller fragments (Andrady, 2011). Pieces of plastic less than 5mm in size are referred to as microplastics (Wright et al., 2013) and these have now become an accumulative problem.

Estuaries are hotspots for microplastic accumulation (Browne et al., 2010; Wright et al., 2013). Galgani et al. (2000) noted that litter, largely plastic, on the seafloor around Europe was most concentrated near estuarine inputs. It is also the case in freshwater catchments that plastic concentrates around water inputs (Corcoran, 2015). Rivers and estuaries receive plastics from terrestrial sources and can transport these to marine systems (Cole et al., 2011; Lechner et al., 2014; Jambeck et al., 2015). For example, it is estimated that over 4 tonnes of plastic

flows into the sea each day from the River Danube (Lechner et al., 2014). Despite this, research has focussed on marine species (Boerger et al., 2010; Foekema et al., 2013; Lusher et al., 2013). There are only a limited number of studies conducted in estuaries (McGoran et al., 2017; Murphy et al., 2017; Bessa et al., 2018).

There are 155 British estuaries, including 35 coastal-plain estuaries (e.g. Thames Estuary; Tinsley, 1998) and 6 fjords (e.g. Firth of Clyde; Jardine, 1986). Reports of plastic pollution in some of these estuaries are escalating. Gallagher et al. (2016) recovered plastics from estuaries in the Solent estuarine complex, Morritt et al. (2014) recorded 8,490 pieces of litter, mainly plastic, during a three-month fyke net fishing programme in the Thames Estuary, and 65% of debris on the shoreline of the Tamar Estuary was found to be in the form of microplastics (Browne et al., 2010).

The Thames Estuary and the Firth of Clyde are comparable with respect to potential plastic pollution: both are in close proximity to several microplastic sources, including major cities and shipping traffic. The 16,000 km<sup>2</sup> catchment of the River Thames includes 15 million residents (Environment Agency, 2016) whilst the River Clyde has a catchment of over 3,000 km<sup>2</sup> which encompasses 1.7 million people (SEPA, 2015).

The Clyde and Thames are ecologically diverse and are important habitats and nurseries for marine fish. The Thames Estuary supports over 950 species, including 112 fish species, and has been recognised as a key habitat for commercial flatfish (Thomas, 1998). The European flounder (*Platichthys flesus*) spends most of its lifecycle in the estuary, and juveniles are able to penetrate the entire tidal reach of the river. Consequently, flounder is a key species to measure the health of the Thames Estuary (Thomas, 1998). Recently McGoran et al. (2017) collected European flounder from two sites in the Thames Estuary to measure the extent of microplastic ingested. The results revealed that up to 75% of sampled *P. flesus* had plastic

fibres in the gut. Scotland's coastline supports ca. 8,000 species (WWF & Scottish Wildlife Trust Joint Marine Programme, 2004), including 59 demersal fish species (The Scottish Government, 2012). The Firth of Clyde is a fjordic system with deep valleys and steep sills (Edwards et al., 1986; Jardine, 1986) and a weak tidal current (less than  $0.5\text{ms}^{-1}$ ; Wilding et al., 2005; The Scottish Government, 2012) which may aid the accumulation of plastics in the sediment which could be available to these demersal species (Haig, 1986). Prior work in the Firth of Clyde revealed that 83% of *Nephrops norvegicus* had ingested plastic (Murray and Cowie, 2011) whilst less than 30% of fish had consumed plastic (Murphy et al., 2017). At present, Murphy et al. (2017) and McGoran et al. (2017) are the only studies to report plastic ingestion by fish in these two estuaries.

The present study extends a preliminary study in the Thames Estuary by McGoran et al. (2017). The aims were to compare (1) samples collected from Thames Estuary and Firth of Clyde fish populations (2) the samples collected in the Thames Estuary to the previous study by McGoran et al. (2017), in which it was found that 20–75% of fish examined had ingested plastic (3) feeding groups and assess if feeding mode affects plastic ingestion in fish and (4) a common prey species (brown shrimp; *Crangon crangon*) with predator fish species. The data collected in this study were also used to determine whether there were any relationships between gender and plastic ingestion.

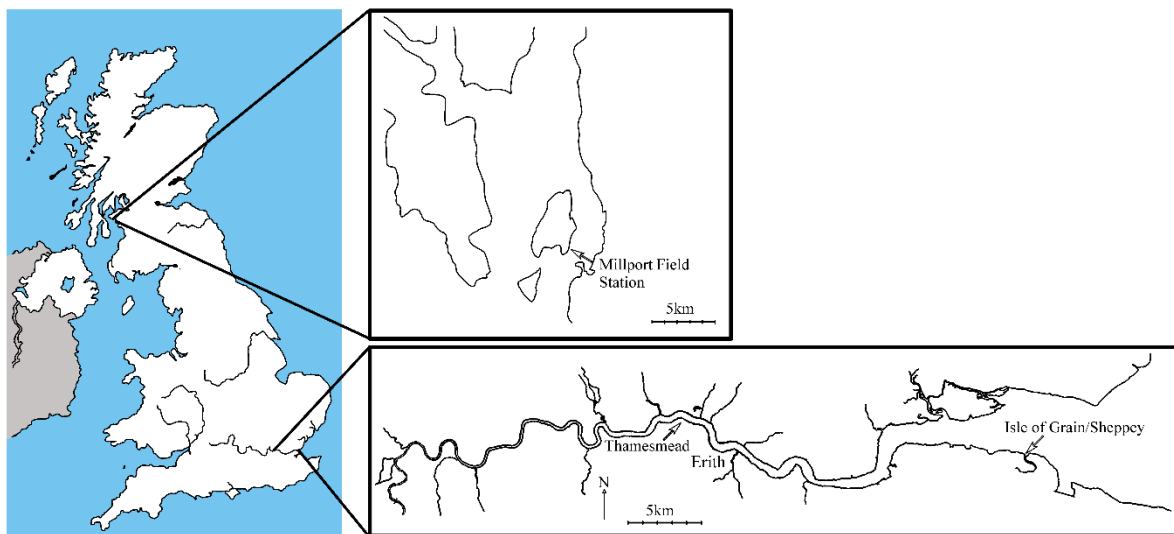
## 2. Materials and methods

### 2.1 Sampling

Using beam trawls (mesh size: 80 mm), fyke, trammel and shrimp nets eight teleost fish species, two cartilaginous fish species, and one shrimp species were caught in the Thames Estuary. Three sampling sites, downstream of London were used: Thamesmead ( $51^{\circ}30.637'\text{N}$   $000^{\circ}06.591'\text{E}$ ), Erith (ca.  $51^{\circ}28.005'\text{N}$   $000^{\circ}12.122'\text{E}$ ) and Isle of Sheppey ( $51^{\circ}29.048'\text{N}$

000°41.800'E; [Fig. 1](#)). Sampling was conducted on 17, 18 and 23 November 2015 at Erith, Thamesmead and Isle of Sheppey, respectively. *Crangon* were not caught from the Isle of Sheppey. Fish were identified, dissected, the gut contents searched and analysed, and blank controls (see section 2.3) collected at Royal Holloway, University of London (RHUL) following the method of [McGoran et al. \(2017\)](#) based on that of [Lusher et al. \(2013\)](#). The resulting data sets are thus directly comparable with these studies.

Fifteen teleost and one cartilaginous fish species were caught in beam trawls (mesh size: 50 mm) in the Firth of Clyde (55°46.240'N 4°52.936'W; [Fig. 1](#)) on 3 November 2015 and 18 May 2016. Fish were identified, dissected, the gut contents searched, and blanks collected, as described above, at Field Studies Council Millport, Isle of Cumbrae and analysed at RHUL. [Appendix A](#) details the sampling sites and equipment used at both sites.



**Fig. 1.** A map of the UK, highlighting the Firth of Clyde (top) and Thames Estuary (bottom) sampling sites. Sampling in the Thames Estuary was conducted at Thamesmead, Erith and Isle of Sheppey.



In total, 876 individuals were examined and 21 species identified (Table B.1; Appendix B). Fish were divided into three functional feeding groups for analysis: flatfish, other benthic fish (excluding flatfish) and pelagic fish. Shrimp were included as a fourth group.

## 2.2 Quantifying plastic ingestion

Samples were transported to the laboratory, stored in a freezer and identified with reference to Wheeler (1978). Prior to dissection, fish were measured (standard length and height), weighed (using a Sartorius 1413 MP8-1 balance accurate to one decimal place or Tesco Go Cook scales accurate to the nearest gram) and any signs of ill-health (i.e. ulcers; Wright et al., 2013) noted. *Crangon* were also measured (length, tip of rostrum to end of telson, and depth of the carapace) and weighed (using Sartorius 1413 MP8-1 balance). No digestion protocols were implemented to reduce the processing time, with some digestions requiring days or weeks (Foekema et al., 2013; Karami et al., 2017; Kühn et al., 2017; Lusher et al., 2017), and to prevent the degradation of polymers that can be caused by many digestive agents (Lusher et al., 2017). The digestive tract from all species was removed and inspected under a dissection microscope using mounted pins. For shrimp, only the foregut was examined for microplastics. The search time was not standardised for this study because of the variability of the size and volume of the digestive tracts from different fish. Searching was conducted in 1 cm sections of the gut thereby reducing its exposure to potential sources of airborne contamination. Any fibres considered to have originated from airborne sources were removed and not included in the examination. Additional controls against contamination are described in section 2.3. Plastic items were removed from specimens and stored on filter paper in a Petri dish sealed with Parafilm. Over 3,000 particles were recovered from the gut contents of fish and *Crangon*.

Gut plastic was initially described by colour and shape. Pale colours, which were difficult to distinguish from one another and fibres without evident pigmentation were grouped

together as “clear fibres”. Several of the potential plastics recovered did not fit into a defined colour category. Plastics with more than one colour were grouped as multi-coloured. Shape was determined as a film, synthetic fibre, sphere or an irregularly shaped fragment.

### *2.3 Controls against contamination*

A clean, white laboratory coat and non-sterile, single-use gloves were worn during dissection procedures and during Fourier Transform Infrared Spectroscopy analyses (see section 2.4). Samples were covered as much as possible to reduce exposure to airborne contamination. Equipment and laboratory space were cleaned with 70% ethanol and white lab roll prior to dissection and searching, as well as between specimens. In addition, empty Petri dishes were placed in each laboratory to monitor environmental contamination. Three replicates were taken, each lasting 30 minutes. Plastics recovered in the Petri dishes were analysed using the methods described for plastics recovered from samples. After FTIR, the limit of detection for each shape and colour plastic was calculated (see below). Plastics were removed from analysis if they did not exceed the limit of detection (LOD). Where the volume of plastic matching the description of a contaminant plastic exceeded that of the LOD, the count was reduced to compensate for contamination (i.e. if the LOD for black fibres was one and a fish ingested three black fibres, only two were reported).

$$\text{LOD} = A + \text{SD}$$

LOD = Limit of detection, A = Average number of plastics of a particular shape and colour (i.e. clear fibres, black films), SD = Standard deviation.

### *2.4 FTIR spectroscopy*

FTIR spectroscopy is well documented for microplastic analysis ([Lusher et al., 2017](#)). [Gallagher et al. \(2016\)](#), however, reported that such analysis is difficult due to the lack of

precise published instructions. As such, detailed methods of FTIR have been included in this paper.

As well as the plastics recovered from the samples, FTIR was conducted on samples of known materials including polyester. As these samples were a known material, it was possible to compare the spectra to the software library ([Appendix C, Table C.1](#)) outputs and ensure that identification using these libraries was accurate. Analysis of plastic pieces was undertaken using a Thermo Scientific Nicolet iS5 FTIR spectrometer, with a diamond attenuated total reflection (ATR) cell and a flat-headed pressure clamp.

All pieces were individually analysed and visible organic matter was removed with a mounted pin before FTIR analysis. A background spectrum was made before analysis and updated hourly. For each individual plastic, 16 scans were collected using Thermo Scientific OMNIC 8.3.103 software, the average result was used to generate an absorption spectrum between 500–4000  $\text{cm}^{-1}$ . This spectrum was compared to 13 standard software libraries ([Appendix C, Table C.1](#)). Identification was informed by [Williams and Fleming \(1995\)](#). Some samples did not precisely match any library spectra and were classified as “spurious results”.

Knotted, woven and networks of fibres were all analysed as a whole rather than individually, with the aim of reducing the loss of fibres before analysis. Fibres from tangled knots were rinsed with distilled water, separated and counted after FTIR.

#### *2.4.1 FTIR data processing*

Thermo Scientific OMNIC Spectra software was used to remove atmospheric  $\text{CO}_2$  absorbance peaks, apply ATR correction, and adjust baselines for 400 spectra. Processed spectra were compared to one software library, the Hummel Polymer and Additives Library. The 400 processed spectra were identified either as the single best match from the spectral library, or from a multiple component match with two spectra.

Composite matches were found for 37% of corrected spectra, 63% of which produced matches with organic matter and a synthetic compound. [Figure D.1b \(Appendix D\)](#) illustrates the output of a multiple component search. The broad peak in the OH region demonstrates that a large quantity of carbohydrate and protein, probably organic matter from the alimentary canal, was present in the sample; other peaks in the spectrum matched polypropylene.

#### *2.4.2 ATR correction*

ATR correction used the following specifications: an angle of incidence of 42°, 1 reflection and refractive index of 1.55 ([Thermo Fisher Scientific, 2015](#)). The refractive index chosen was an average of refractive indices of three common polymers (nylon 6, polyester and polypropylene) provided by [Greaves and Saville \(1995\)](#), which ranged between 1.496 and 1.706. ATR correction using a refractive index between 1.50 and 1.60 showed minimal variation in the output spectra. ATR correction increased the match of a known polyester sample by 20% compared to atmospheric and baseline correction alone.

[Figure D.1a \(Appendix D\)](#) shows the processed FTIR spectrum obtained from a clear fibre. Peaks in the fingerprint region closely match polypropylene. The sample spectrum and library spectra did not match perfectly as the sample had been degraded in the environment and / or in the fish gut. ATR correction did not increase the average percentage match of the sample with library spectra ( $41 \pm 14.6\%$  before and  $37 \pm 20.2\%$  after) but did increase the maximum recorded match from 87% to 97%. ATR correction, on average, increased the accuracy of a match by 5.3 percentage points, and increased the apparent percentage of synthetic spectra in the sample by 21 percentage points to 58% of the sample. Since a higher percentage match was obtained from corrected spectra, these were used for data collection.

#### *2.5 Statistical Analysis*

Statistical analysis was conducted using R version 3.4.2 with R Studio version 1.1.383. Generalised linear models (GLMs) were developed to understand the variables that influenced the number of organisms to ingest plastic and the number of plastic pieces by individuals. The season of sampling, length of organism, gender, feeding group and sample site were investigated. GLMs were compared with AIC and BIC scores so that only reduced models with the main effects were used for analysis. Non-significant variables were removed until eight GLMs were generated (Table 1). Length was skewed, with a higher number of smaller specimens sampled than larger ones. To account for this, length was transformed in the models. To analyse the number of organisms to ingest plastic, binomial models were used. To compare the amount of plastic ingested by individuals, specimens that ingested no plastic were removed from analysis. Shrimp were excluded from comparisons between the Thames and the Clyde as they were only sampled from the Thames. Seasonality could only be considered when analysing fish from the Clyde as Thames samples were all collected in winter. When comparing gender, only the most common flatfish species at each site were analysed. When categorical variables were significant, the results were interpreted using Tukey pairwise comparisons. Similarly, when interaction terms were significant, the results were interpreted by model reductions and ANOVA comparisons.

### **3. Results**

#### *3.1 Contamination*

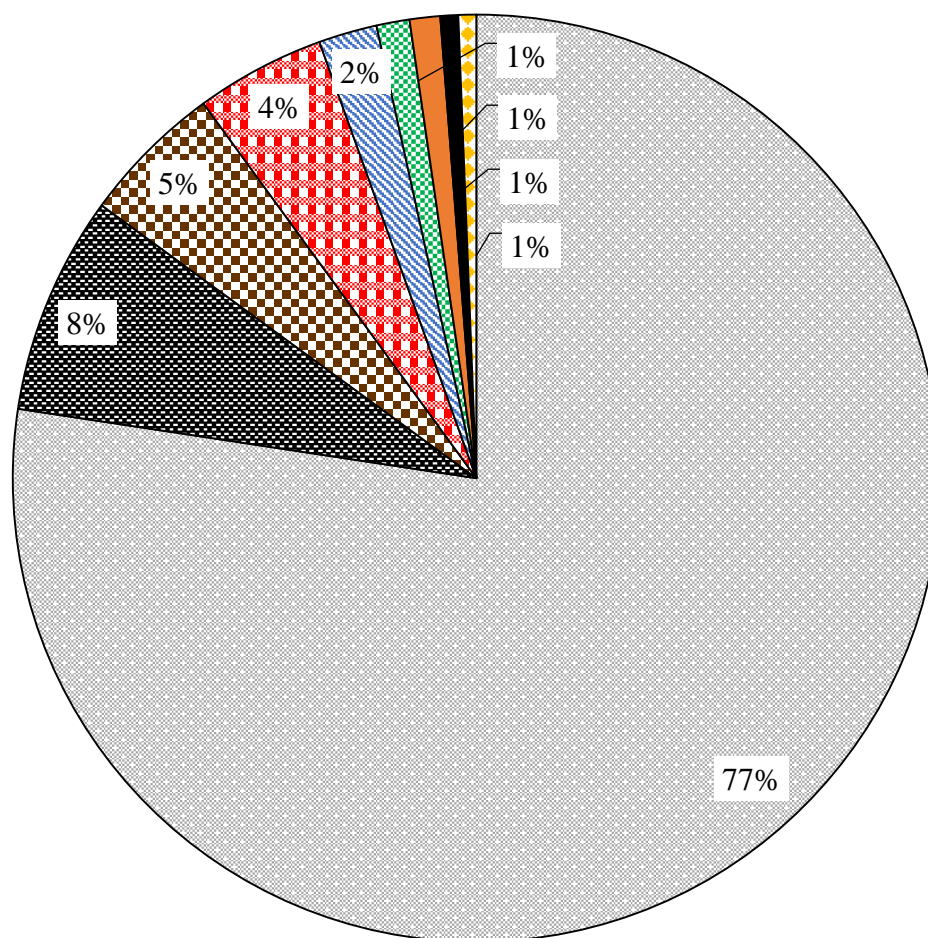
Airborne contamination was reported in both laboratories (see Table 2). Fibres were identified as cotton and polyester; films were also identified as polyester. All clear, red, black or blue fibres and black films at or below the LOD were removed from analysis.

#### *3.2 Plastic abundance*

Prior to FTIR, 3,427 potential plastic pieces (Thames Estuary: 850; Firth of Clyde: 2,577) were collected. Fibres were the most abundant plastic, occurring as single filaments and tangled knots. Spheres, films (including sheets of woven fibres), fragments and joined networks of filaments were also recovered.

Fibres lost or destroyed prior to FTIR could not be analysed. FTIR was conducted on the remaining 2,649 particles. Of this subset, 1,285 (48.5%) were confirmed to be synthetic by FTIR analysis when compared to library spectra of known polymers. This volume decreased to 1,128 pieces of plastic when contamination was considered. Among these samples, 26 different polymers and polymer mixes were identified ([Appendix E](#)). The most common polymers were polyester (polymers grouped together; 33%), nylon (polyamide 6 + polyamide 6.6; 20%) and polypropylene (15%).

A variety of coloured plastics were collected, 12 in total ([Fig. 2](#)). Tangled knots that contained more than one colour fibre were treated as multi-coloured. The most commonly recorded colours were clear (77% of plastics), black (8%), brown (5%) and red (4%).



clear
  black
  red
  brown
  blue
  green
  other colours
  yellow
  multi-coloured

**Fig. 2.** The colours of plastics recorded from both estuaries and all samples. Other colours included grey, white, purple, orange and pink.

Tangled fibres were recorded in 31 specimens (7: Thames Estuary; 24: Firth of Clyde) with only two fish, both from the Firth of Clyde, containing more than one knot. Tangled knots ranged from 2–51 fibres per knot. Tangled knots contained 1–5 colours, but most comprised only one, commonly clear fibres.

### 3.3 Plastic prevalence in fish and shrimp

After FTIR analysis, the number of fish species (both benthic and pelagic) that had ingested plastics was confirmed as 13 (Thames Estuary: 8; Firth of Clyde: 6). Plastic was also ingested by brown shrimp (*Crangon crangon*). Overall, 32% of estuarine organisms (36% of fish and 6% of *Crangon*) had ingested plastic, a total of 278 individuals.

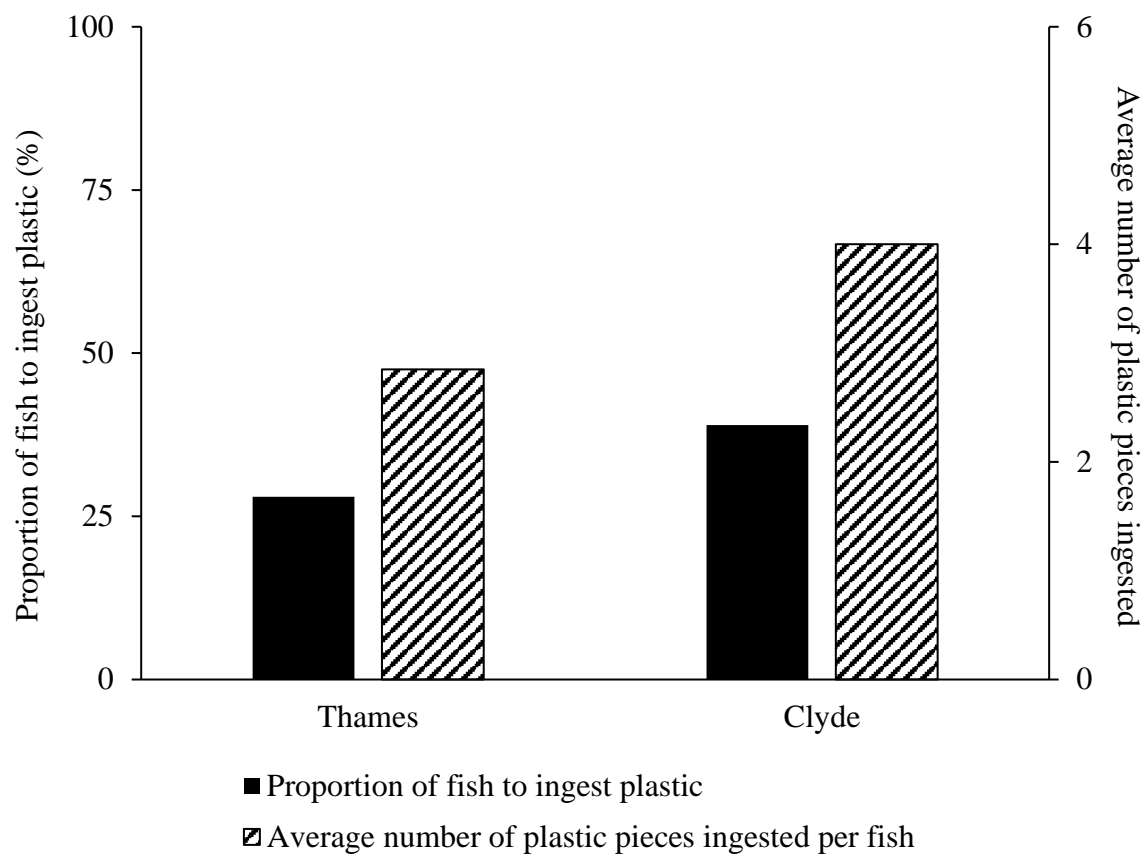
Table 3 shows the proportion of individuals to ingest plastic and the average consumption of plastic in each feeding group. In the Thames Estuary, 33% of flatfish, 19% of other benthic fish, 14% of pelagic fish and 6% of *Crangon* ingested plastic. An average of 2.93, 1.50 and 3.20 plastic pieces were ingested by Thames flatfish, other benthic fish and pelagic fish, respectively. The most common polymer recovered was nylon, which made up 33% of recovered plastics. In the Firth of Clyde, 39% of flatfish, 14% of other benthic fish and 60% of pelagic fish ingested plastic. On average Clyde flatfish, other benthic fish and pelagic fish ingested 3.92, 2.00 and 5.83 plastic pieces respectively. In Clyde fish, polyester was the most common polymer (37% of plastic pieces).

At Thamesmead, Erith and Isle of Sheppey 18%, 34%, and 32% of fish ingested plastic, respectively. An average of 2.2, 3.6 and 1.6 particles were ingested per fish at each site respectively. Comparatively few *Crangon* ingested plastic: 7% and 5% of *Crangon* from Erith and Thamesmead, respectively. The most common polymer recovered from *Crangon* was nylon (43% of plastic pieces). An average of 0.07 and 0.05 pieces of plastic were recorded in the stomach of Erith and Thamesmead *Crangon*, respectively.

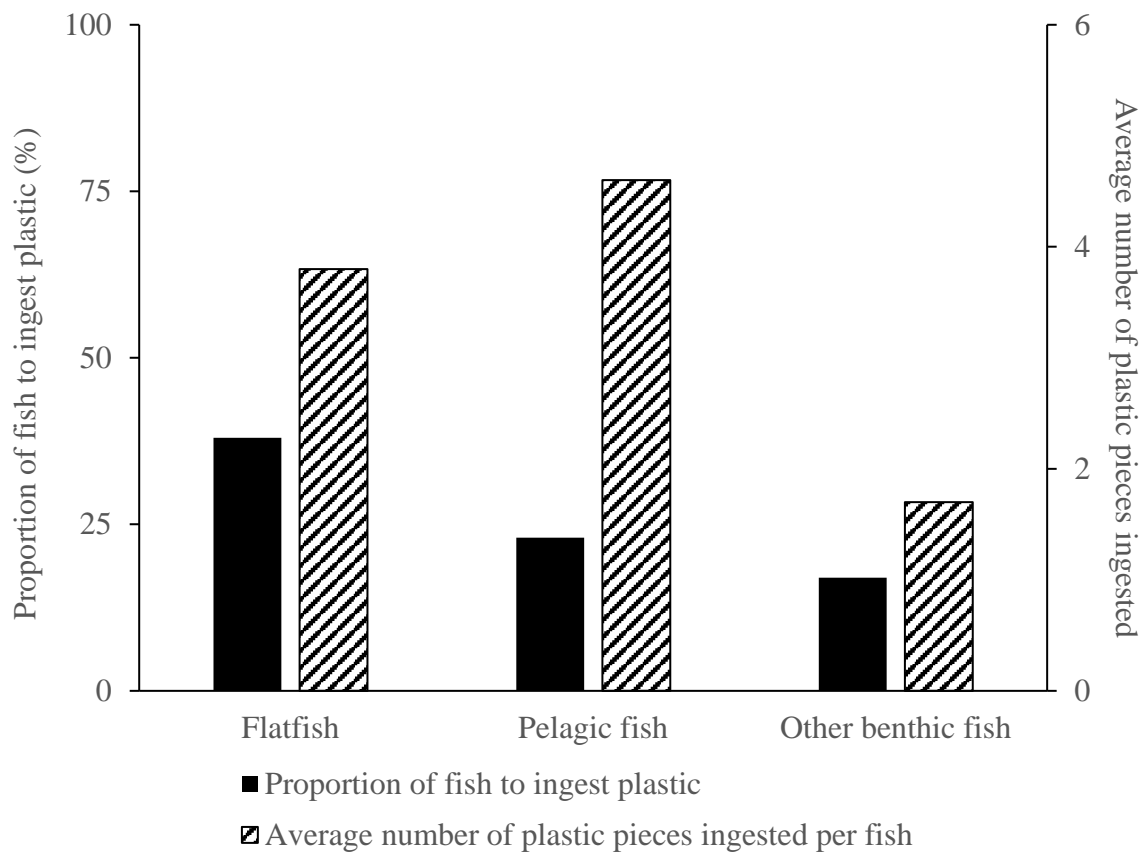


### 3.4 Statistical Analysis

Generalised Linear Model (GLM) 1 and 5 (Table 1) included fish from both the Thames and the Firth of Clyde. The models revealed that significantly more fish from the Firth of Clyde ingested plastic than from the Thames ( $p < 0.001$ , 39% of fish from the Clyde ingested plastic compared to 28% of fish from the Thames; Fig. 3). The average number of plastic pieces ingested by fish, however, did not differ between the two sites. Additionally, significantly more flatfish ingested plastic than other benthic fish ( $p < 0.05$ , 38% of flatfish ingested plastic compared to 17% of other benthic fish; Fig. 4). Also, other benthic fish ingested significantly less plastic than both pelagic fish and flatfish ( $p < 0.001$ , on average flatfish ingested 3.8 pieces of plastic, pelagic fish ingested 4.6 pieces and other benthic fish ingested 1.7 pieces; Fig. 4). Analysis of the amount of plastic ingested by individual fish demonstrated that length only influenced the number of plastic pieces ingested and not the number of fish which consumed plastic. Larger fish ingest significantly more pieces of plastic ( $p < 0.001$ , slope: 1.1384, intercept: -3.9373).



**Fig. 3.** A greater proportion of fish from the Clyde ingested plastic when compared to fish from the Thames. The average number of plastic pieces ingested per fish did not differ between the sites.

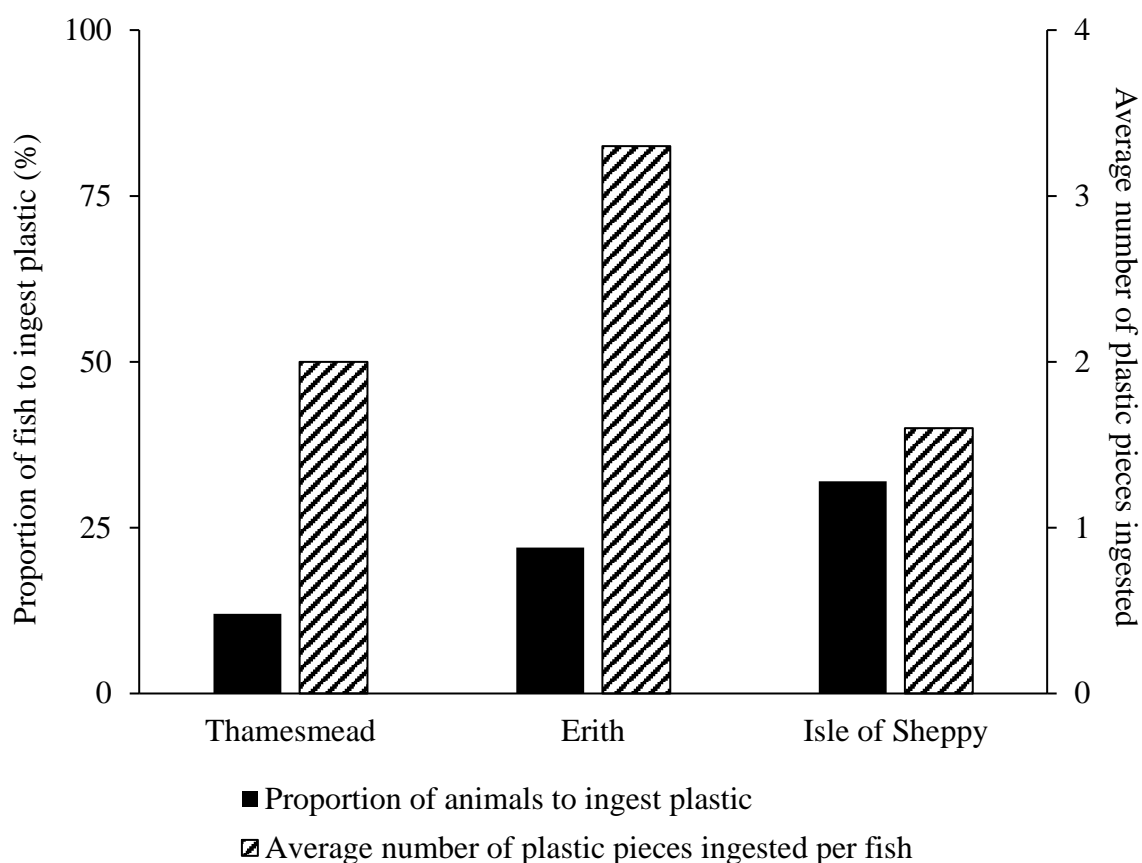


**Fig. 4.** A greater proportion of flatfish ingested plastic than other benthic fish, but not pelagic fish. Both flatfish and pelagic fish ingested, on average, more pieces of plastic than other benthic fish.

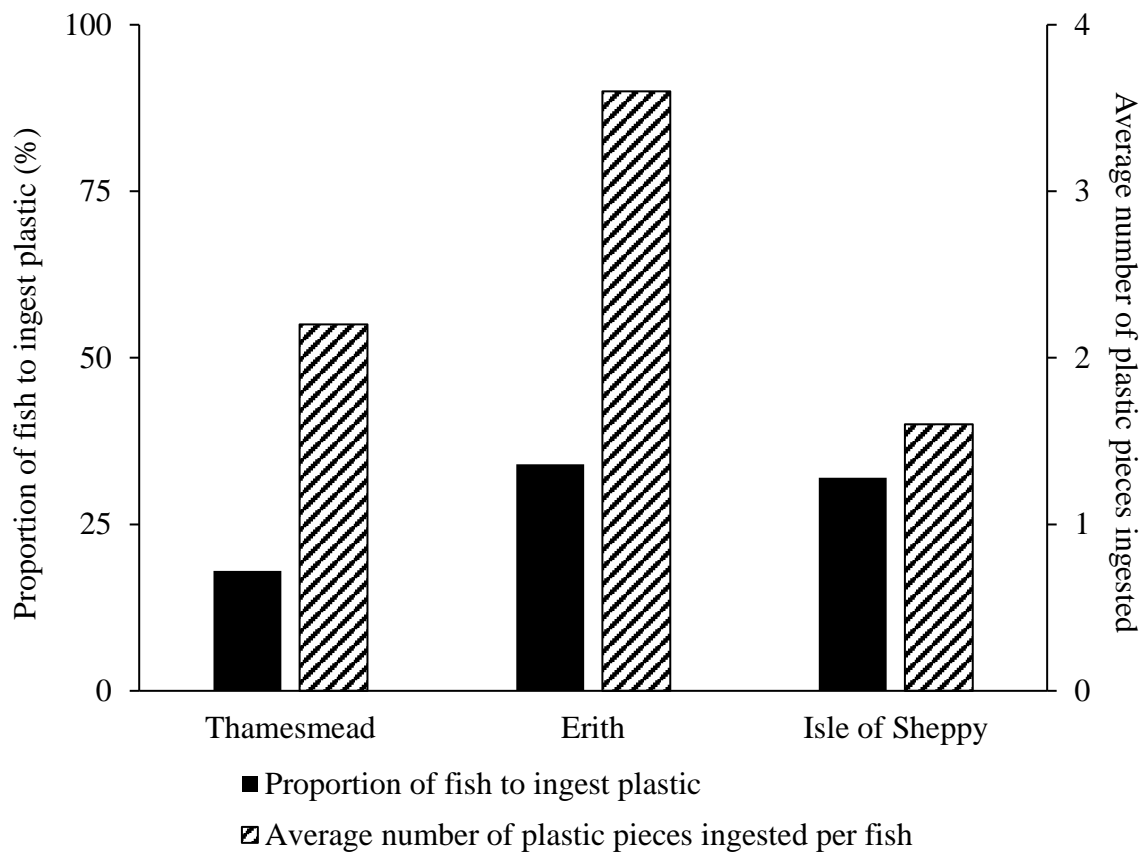
For model 2 and 6 (Table 1), Thames individuals were analysed separately from Clyde fish. This allowed for the analysis of shrimp (only collected in the Thames) and the subsampling sites in the Thames (Thamesmead, Erith and Isle of Sheppey). A greater proportion of animals from Erith ingested plastic compared to Thamesmead ( $P < 0.05$ , Erith: 22%, Thamesmead: 12%; Fig. 5). Animals sampled from Erith also ingested more pieces of plastic on average ( $p < 0.05$ , the average plastic ingestion per individual from Erith was 3.3 pieces, compared to 2 in Thamesmead and 1.6 in the Isle of Sheppey; Fig. 5). Model 2 revealed that

302 larger organisms in the Thames tended to ingest plastic ( $p < 0.001$ , the average length of  
 303 organisms to ingest plastic was 221.7 mm compared to 131.8 mm for organisms that did not  
 304 ingest plastic). In the Thames, a greater proportion of flatfish ingested plastic than other benthic  
 305 fish, the other feeding groups did not significantly differ from each other ( $p < 0.05$ , 33% of  
 306 flatfish in the Thames ingested plastic compared to 14% of pelagic fish, 19% of other benthic  
 307 fish and 6% of shrimp). Pelagic fish and flatfish also ingested more pieces of plastic than  
 308 shrimp did ( $p < 0.05$ , average plastic ingestion by flatfish was 2.9 pieces, for pelagic fish was  
 309 3.2 and for other benthic fish was 1.5).

A



B



**Fig. 5.** The amount of A) animals (including both fish and shrimp) and B) fish (only) to ingest plastic and the average amount of plastic ingest by individual was greatest at Erith.

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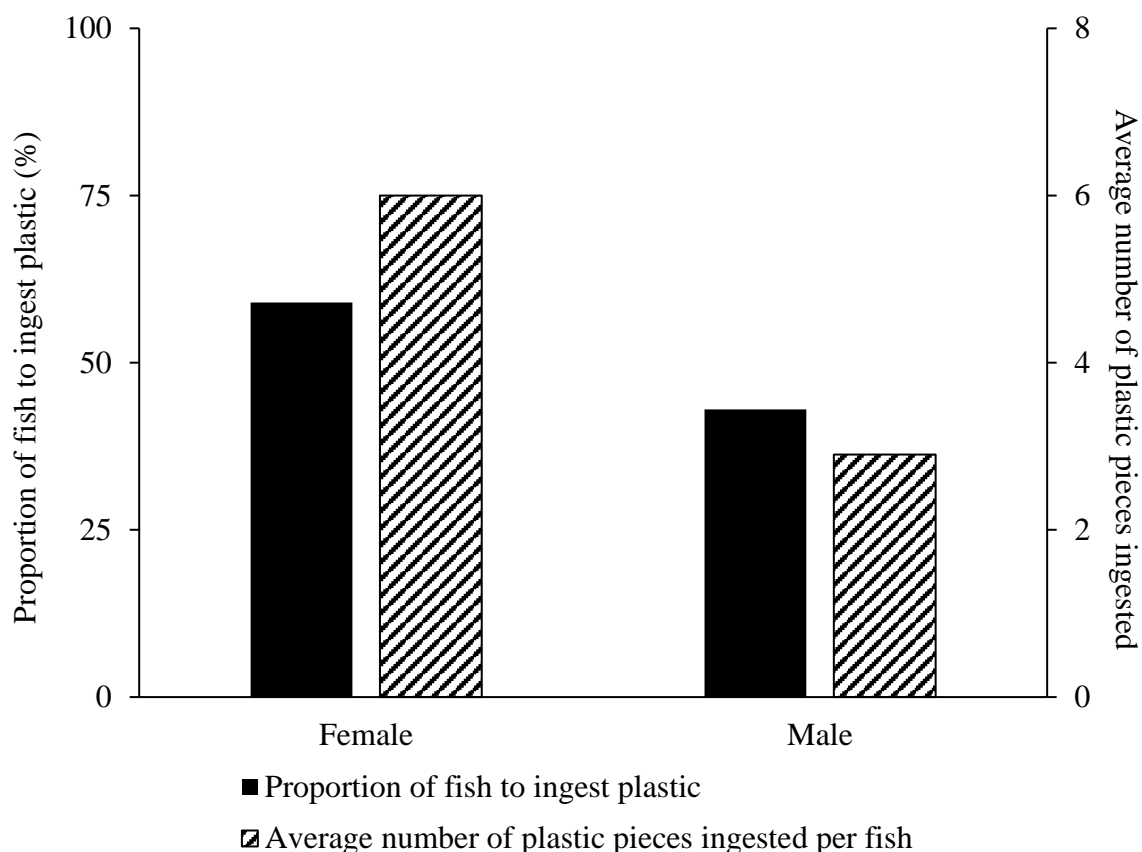
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Models 3, 4, 7 and 8 (Table 1) compared the number of flounder in the Thames and dab in the Clyde found to ingest plastic as well as the amount of plastic ingested by these species. The models highlighted differences in the significance of gender on plastic ingestion. For Thames flounder, gender did not significantly affect the number of fish found to ingest plastic or the number of plastic pieces ingested ( $p > 0.05$ ). However, in the Clyde, 59% of female dab ingested plastic compared to 43% of males ( $p < 0.05$ ; Fig. 6), on average ingesting 6 pieces of plastic and 2.9 pieces, respectively ( $p < 0.01$ ; Fig. 6). The season of sampling also had a significant impact on plastic ingestion by Clyde dab. A higher proportion of fish sampled

319 in the summer ingested plastic ( $p < 0.001$ , 71% compared to 6% in winter). Additionally, fish  
 320 from the summer samples ingested an average 4.6 pieces of plastic per individual compared to  
 321 1.2 pieces ingested by winter fish ( $p < 0.05$ ).



**Fig. 6.** More female dab, *Limanda limanda* (Linnaeus, 1758), in the Firth of Clyde ingested plastic than males; females also consumed significantly more plastic on average.

322

## 323 4. Discussion

### 324 4.1 Polymer diversity

325 The types of polymers recovered varied between the Firth of Clyde and the Thames  
 326 Estuary. Nylon was the most abundant polymer recovered from the Thames Estuary whilst  
 327 polyester was the most abundant polymer in the Firth of Clyde. Both nylon and polyester are  
 328 used in the textile industry. Nylon is also used in fishing industry and polyester is a major

component of wet wipes. Products and by-products of these industries could be responsible for much of the pollution in the Thames Estuary and Firth of Clyde. Indeed, [Thames21 \(2018\)](#) have recovered over 5,450 wet wipes from the foreshore of the Thames. Despite the difference in polymer type between the sites, the colours recovered were the same at both sites. Clear fibres were the most abundant followed by black plastics.

#### *4.2 Limitations of FTIR*

Nylon samples produce similar spectra to those of organic polyamides and there is a possibility of misidentification of both nylon and organic samples. If all nylon samples were removed from this study, a minimum of 33% of fish would have ingested plastic – a similar proportion of ingestion was reported in freshwater fish in the Thames ([Horton et al., 2018](#)). The statistical analyses used in the present study assume that FTIR is accurate in its identification of this synthetic polymer. Micro-FTIR had it been available in the present study, would have likely increased the proportion of fibres accurately matched with library spectra.

#### *4.3 Plastic ingestion*

A lower proportion of fish from both sites ingested plastic than in the preliminary study ([McGoran et al., 2017](#)), 36% compared to up to 75%. However, plastic was ingested by fish at all sites and in all feeding groups, including previously poorly studied fish, such as elasmobranchs ([Smith, 2018](#)). By including a greater diversity of species and a larger sample size, the present study may better represent the state of plastic pollution in UK estuaries, but it must also be noted that the methodology used in both studies only provides a snapshot of the situation and that plastic ingestion may fluctuate. It is important to be aware that, in both the Thames and the Clyde, sample size for pelagic fish and other benthic fish was relatively low (other benthic: 21 in Thames, 14 in Clyde; pelagic fish: 37 in Thames, 10 in Clyde). This could impact the strength of statistical analysis.

Larger individuals ingested more plastic than smaller ones irrespective of site of origin, feeding group or gender. This is to be expected as larger animals will have greater energetic requirements and will require a greater intake of food, increasing their chances of consuming plastic. It may also be possible that the prey items of larger individuals, which differ from that of smaller specimens (Schückel et al., 2012), are visually more similar to the plastics and are, as such, ingested more frequently in larger fish. Schückel et al. (2012) reported that as dab (*Limanda limanda*) and plaice (*Pleuronectes platessa*) grew, their diet focussed on larger prey, such as polychaetes. Prey selection with regards to plastic ingestion was not examined in the present study and is a topic for future analysis.

Although only a few species were present in both the Thames and the Clyde, the similarity in their feeding strategies was used to overcome the differences in species assemblages. Overall, more fish from the Firth of Clyde ingested plastic than in the Thames, but the average number of plastic pieces ingested by fish did not differ between sites. Estuaries are complex systems and it is not possible in the present study to determine the factors which influence plastic ingestion. But, the Clyde and Thames represent different types of estuary (fjord and coastal-plain respectively) and geological and hydrodynamic factors are likely having an impact. Fjordic systems are defined by deep valleys in the riverbed. These ridges may capture and accumulate microplastics. The Clyde was dominated by flatfish species, which could be exposed to large quantities of plastic on the benthos. Bottom water in the Firth of Clyde takes ca. 1 month to pass out to sea (Edwards et al., 1986) and plastics may therefore be retained in the sediment for a long time. The slower flowing waters in the Clyde may therefore help to explain why more plastic was consumed by fish in this catchment compared to the Thames Estuary.

As estuaries are routes to the sea, it could be argued that microplastics should accumulate downstream in the estuary (Isle of Sheppey). Indeed, Lee et al. (2013) found this



to be the case and [Browne et al. \(2010\)](#) noted that the high flow rate and turbulence in estuaries can keep high-density plastics suspended until they reach the sea. In the Thames, however, both a greater proportion of fish ingested plastic and the average amount of plastic ingested was greater in fish from Erith than in fish from Thamesmead and the Isle of Sheppey. Similarly, [Morritt et al. \(2014\)](#) also found that plastic in the Thames did not move downstream. The tidal nature of the Thames Estuary may lead to long-term upstream retention of microplastics. Additionally, plastic abundance increases with proximity to urban centres ([Barnes et al., 2009](#); [Corcoran, 2015](#)). Perhaps a combination of the movement of plastics downstream and the tide pushing plastics upstream has resulted in plastics accumulating downstream of London and Thamesmead at Erith, but upstream of Sheppey. Additionally, a greater number of wastewater treatment plants are present near Erith ([Westlake, 2016](#)). Wastewater effluents are well documented as large microplastic inputs to rivers (as discussed in section 4.5). Large waste tips in the area could also be responsible for some of the plastic entering the estuary.

More flatfish ingested plastic than other benthic fish, which could highlight differences in their feeding strategies which makes flatfish more prone to ingesting plastic. It is possible that plastics may accumulate in the sediment; thus, plastic may be more available to bottom-dwelling organisms. Flatfish are closely associated with the sediment and can act as ambush predators. Some flatfish species are known to consume sediment with their prey ([Hurst et al., 2007](#)). This could be a route of exposure to microplastics. Flatfish may mistake plastic, especially fibres for prey, such as bivalve siphons and polychaetes. The digestive tracts of other benthic fish were also found to contain less plastic than those of pelagic fish and flatfish, but it must be noted that this is based on a small sample size of only 35 benthic fish. In the Thames, 33% of flatfish ingested plastic whilst only 14% of pelagic fish and 19% of other benthic fish consumed plastic. [McGoran et al. \(2017\)](#) also reported that flatfish in the Thames Estuary ingested more plastic than pelagic fish. In comparison however, some studies have reported no

such difference (Lusher et al., 2013). Analysis of sediment samples could provide evidence for the retention of plastics in such deposits and go some way to explaining plastic ingestion in flatfish.

The density of plastics, currents, turbulence, inflows, seabed topography and hydrodynamics determine the depth distribution of plastic pieces in the water column (Cole et al., 2011). High-density plastics and plastics coated in biofilms occur lower in the water column and are expected to be prominent in the diet of benthic fish (Barnes et al., 2009; Cole et al., 2011; Corcoran, 2015). In this study, polyvinyl chloride, acrylic and polyester, all of which are high-density plastics, were found exclusively in flatfish. On the other hand low-density microplastics (e.g. polyethylene and polystyrene), along with those plastics re-suspended by turbulence, float near the water surface and are available to smaller organisms such as plankton (Cole et al., 2011) as well as pelagic fish. Polystyrene (and polystyrene mixes) and polyethylene were more abundant in benthic species. It is possible that consumption of plankton by invertebrates or fish results in trophic transfer and bioaccumulation. Thompson et al. (2004) proposed that polymer density does not influence the distribution of plastics, recording various polymers in both the water column and the sediment. Many other factors can influence the distribution of plastics. For example, plastic density can be impacted by biological and environmental factors such that plastics can flocculate, increasing density, and sink (Barnes et al., 2009). Song and Andrady (1991) reported that biofouling aids the sinking of plastics in the marine environment. Tangled knots were only recorded in flatfish, as they likely sank with the combined density of the fibres. Alternatively, they may have formed in the stomach of the fish. In *N. norvegicus*, most ingested plastic was tangled filaments, and Murray and Cowie (2011) suggested these balls originated from the sediment or were ingested through trophic transfer. In addition, knots of fibres were observed in the gastric mill of Chinese mitten crabs, *Eriocheir sinensis*, from the Thames (Emma Powell, pers. comm.) and perhaps formed due to

the action of the gastric mill. Native crab species were observed in the diet of flounder and were perhaps a source of knotted fibres.

Female dab in the Clyde ingested more plastic than the males, but this trend was not seen in Thames European flounder. [Horton et al. \(2018\)](#) also found that female freshwater fish in the UK ingested more plastic than males. It has been suggested that water quality may impact plastic ingestion differently for males and females, and that females may have larger energy requirements than males; leading to increased food consumption and greater exposure to plastic ([Horton et al., 2018](#)). Likewise, [Vassilopoulou and Haralabous \(2008\)](#) reported that female flatfish have a lower condition factor than males, especially during the breeding season, due to greater energy demands. Additionally, female dab grow quicker than males ([Wheeler, 1969](#)) and may metamorphose earlier, becoming a substrate feeder sooner and having a higher exposure to plastics. Females also take an additional year to become sexually mature ([Wheeler, 1969](#)), which could enable them to forage more whilst males compete for mates. [Schückel et al. \(2012\)](#) demonstrated that diet changes as fish grow. It is possible that female dab, which grow quicker, may be switching to a prey source that is visually more similar to plastic, such as polychaetes, sooner than males. Additionally, size differences between dab and flounder may facilitate resource partitioning, resulting in differences in plastic ingestion. On average, Thames flounder ingested 3.1 pieces of plastic, whilst dab from the Clyde ingested a mean of 4.5 pieces of plastic ([Table B.1, Appendix B](#)). Differences in the behaviour of flounder and dab may explain why only dab demonstrated variation in ingestion between genders. [Hurst et al. \(2007\)](#) reported that the foraging behaviour of three co-existing flatfish species, which occupy the same ecological guild, was determined by distribution, foraging times, habitat use and differences in prey. The diets of flounder and dab differ, despite the species sharing some common prey items. Adult flounder have a mostly mollusc-based diet, whereas dab have a wider diet that mostly consists of crustaceans and polychaetes ([Wheeler, 1969](#); [Schückel et al.,](#)

2012). Each niche could result in different exposures to plastic pollution. Although dab and flounder are present in both the Thames and the Clyde, they are not equally abundant at both sites. This makes comparisons difficult. Additionally, summer samples were collected from the Firth of Clyde but not from the Thames. Seasonality had a significant impact on plastic ingestion in the Clyde, with a much higher proportion of fish in summer ingesting plastic, on average almost 4 times as much in winter. The same might have been true in the Thames. Vassilopoulou (2006) found that the diet of *Lepidorhombus boscii* varied seasonally, with individuals ingesting less material but a greater variety of prey items during winter and spring compared to summer and autumn. This could result in variations in the amount or variety of plastic ingested. Wheeler (1969) noted that flounder is a more active feeder during the warmer months and in mid-winter can almost completely stop feeding. Additionally, both dab and flounder spawn between February and June (Wheeler, 1969). By sampling dab in May, the present study may have highlighted variation in foraging behaviour during the spawning period. Too few trawls were taken, however, to accurately identify temporal differences in plastic ingestion. Gender differences in plastic ingestion could not be fully explained in this paper and are a subject for future analysis.

#### 4.4 Impacts of plastic ingestion

Although plastic ingestion has the potential to cause many ill effects to aquatic organisms, including abrasions, ulcers, false satiation and blockages in the digestive tract (Wright et al., 2013), previous experiments into the effects of microplastics have used unrealistic concentrations. Additionally, it is likely that many microplastics are passed through the alimentary canal of a fish without complication (Jovanović et al., 2018). Large prey items, such as shrimp and bivalves were found to have been ingested by most fish and it is likely that plastics would be egested with waste remains of these prey items. No tangled fibres were large enough to cause blockages or lead to false satiation since prey items bigger than the knots were

recorded. Grigorakis et al. (2017) demonstrated that neither microbeads nor microfibres were retained in the gut longer than digesta, concluding that microplastics did not accumulate in the gut over successive meals. Gut morphology is known, however, to impact the retention of plastics (Jabeen et al., 2017). Equally, Jovanović et al. (2018) reported that virgin microplastics did not accumulate in the alimentary canal of adult fish. After 45 days of exposure to microplastics no stress or ill-effects were reported.

#### 4.5 Trophic transfer

In the present study, pelagic fish and flatfish ingested more plastic than shrimp. This could be indicative of bioaccumulation in the food chain. Plastics available to lower trophic level organisms, such as crustaceans and bivalves, could also be accessible to higher trophic level organisms, such as fish, through ingestion. Welden and Cowie (2016) found that *N. norvegicus* ingested plastic and reported that an average 74% of their diet consisted of crustaceans and bivalves. Similarly, *C. crangon* feed on a range of organisms including molluscs (Devriese et al., 2015) and it is well documented that molluscs ingest plastics (Van Cauwenberghe and Janssen, 2014; Van Cauwenberghe et al., 2015). Research by Farrell and Nelson (2013) suggests that the plastic load of molluscs could be passed on to *Crangon* via ingestion. Welden and Cowie (2016) reported that smaller langoustine retained more plastic in the foregut, likely due to the morphology of the gastric mill plates. It is possible that the same is true for *Crangon* and that plastics from the shrimp could potentially be transferred to the fish. Brown shrimp make up a large part of the diet of the two most common fish in the present study, dab and flounder (Wheeler, 1969), and were found in the gut of many of the fish in this study (including flounder, pouting, sole, whiting, roker and eel).

In this study, 6% of *Crangon* ingested plastics, whilst a much higher proportion of fish ingested plastics (36%). The difference in consumption of plastic by fish and shrimp may be

due to bioaccumulation. In a study by [Devriese et al. \(2015\)](#), 63% of *Crangon* ingested plastics, which could result in a high plastic exposure for fish through their diet. Furthermore, the acid digestion protocol used by [Devriese et al. \(2015\)](#) may have recovered plastics that were left undiscovered in the present study, where only the stomach was searched. In fact, [Devriese et al. \(2015\)](#) reported the multipart intestinal tract of *Crangon* as a key factor in the storage of plastics. Future analysis of *Crangon* should use a digestion protocol or investigate the whole digestive system to ensure that all microplastics are recovered. Moulting stage, size and sex also impact plastic retention in crustaceans ([Welden and Cowie, 2016](#)). Shellfish, including the white furrow shell, *Abra alba*, and polychaetes were also recorded in the diets of flatfish in the present study and the literature ([Wheeler, 1969](#)), whilst cod, dogfish and eels all ingested fish, in some cases whole. Dab have also been recorded as feeding on fish ([Wheeler, 1969](#)). While evidence of dogfish (*Scyliorhinus canicula*) ingesting plastic is limited. The present study produces an estimate of ingestion in line with the only other published study to include this species: 14% compared to 15% by [Smith \(2018\)](#). As an opportunistic predator, feeding on crustaceans and fish, as well as many other prey items, dogfish could be exposed to plastic through their prey and through the water column. The present study indicates that fish consume plastic and previous studies have established plastic ingestion in bivalves and polychaetes ([Van Cauwenberghe et al., 2015](#)). Trophic transfer has been demonstrated in laboratory studies ([Murray and Cowie, 2011](#); [Farrell and Nelson, 2013](#); [Watts et al., 2014](#)), suggesting that these could act as potential sources of plastic for predatory fish species. It should be noted that trophic transfer may be having a minimal effect on plastic retention ([Chagnon et al., 2018](#)).

#### 4.5 Sources of plastic

The Clyde and Thames Estuaries are major shipping channels ([Port of London Authority, no date](#)). The considerable maritime traffic associated with these two catchments potentially results in significant inputs of plastic litter from shipping ([Haig, 1986](#); [Tivy, 1986](#)),

which could break down and account for many of the fibres recorded in this present study. In the Adriatic Sea, 53% of plastic originated from fisheries and the aquaculture industry (Strafella et al., 2015). Cole et al. (2011) reported that fishing gear, typically made of nylon, could be found at variable depths in the sea, becoming available to both pelagic and benthic fish. This was supported by the present study, which found that benthic fish, including flatfish, and pelagic fish had ingested nylon. In addition, *Crangon* had also ingested nylon filaments. Plastic fibres have also been linked to sewage works (Dubai and Liebezeit, 2013; Free et al., 2014), as shown by Browne et al. (2011) who reported that sites which have been used for sewage disposal contained 250% more plastic, mostly fibres, compared to locations which did not have sewage deposits. Many wastewater treatment plants do not have filters small enough to remove microplastics (Mourkogiannis et al., 2018). Some of these plants release only a small number of particles per litre of effluent (Ziajahromi et al., 2017). However, the large volumes of effluent released result in many thousands of particles entering waterways. Additionally, particles removed from wastewater accumulate in sewage sludge, tens of thousands per kg of dry sludge (Li et al., 2018). This sludge is often applied to agricultural land, providing both a route to terrestrial systems, but also back into aquatic systems through run off. The sewage works in the Thames Estuary are likely responsible for many of the fibres in this study. Browne et al. (2011) found a similar abundance of synthetic fibres in wastewater effluents at sites used for sewage disposal and expected that these originated from washing machine outputs. The researchers also demonstrated that some textiles can shed nearly 2,000 fibres per wash (Browne et al., 2011).

Woven fibres were identified as polypropylene by FTIR, although some produced spurious results. These likely originate from larger sources (e.g. sanitary products, which are abundant in the Thames; Morritt et al., 2014). Plastic films also originate from larger sources, such as carrier bags.

In conclusion, this study reveals that plastics are ingested by both benthic and pelagic fish populations from two UK estuaries, although to a lesser degree than expected, when compared to McGoran et al. (2017). More fish from the Clyde and more flatfish ingested plastic. Our results highlight the severity of estuarine plastic pollution in the UK and the need for more research into freshwater and estuarine ecosystems.

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## **Author Contributions (initials in alphabetical order)**

ARM, DM and PRC conceived and designed the study, with ARM and PFC undertaking data collection. ARM and JPM analysed the data. Materials and analysis tools were contributed by JPM and PRC. The paper was written and approved by all authors: ARM, DM, JPM, PFC, PRC.

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